Colonies heterozygous at the 2 loci B and R were test-crossed to double recessive colonies (bbrr). The larvae were collected directly from the donor; at the time of metamorphosis they were attached to glass slides; the new colonies derived from them were reared in aquaria and scored for pigmentation characters.

Results. The distribution of the phenotypes in the offspring of 6 matings is illustrated in the table. The segregation of the alleles at either locus fits the expected 1:1 ratio. The joint segregation reveals a tight linkage between B and R, with the dominant alleles in the repulsion phase. Actually, all but one of the heterozygous parental colonies were derived from 2 matings of the type Br/br × bR/br.

For the total series, the recombination value is 1.94% ± 0.78. The 2 partial series derived from heterozygotes in female and male phase, respectively, were 1 BR, 79 Br, 90 bR, 3 br (total 173), and 2 BR, 64 Br, 70 bR, 0 br (total 136); they have similar recombination values of 2.31 and 1.47. The parental genotypes in family No. 5 were AaBbRr and aabbrr. The joint segregation of A–B (17 AB, 12 Ab, 12 aB, 11 ab) and A–R (12 AR, 17 Ar, 11 aR, 12 ar) agrees with the previously established independence of locus A from both B and R.

Discussion. Little is known of the genetics of ascidians. The colonial species Botryllus schlosseri has proved to be a suitable material which can be easily reared under controlled breeding conditions in the laboratory, where it gives clones that can be maintained for years. In addition to colour polymorphism, other characters are now under study. The linkage between the loci B and R, with recombination in both sexes, is the first to be discovered and represents the nucleus of the first linkage group within the haploid set of 16 chromosomes of this species[6].

[1] Investigation supported by a grant from C. N. R. through the Institute of Marine Biology of Venice. The author is grateful to W. J. Canzonier for the correction of the English manuscript.

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Considerations of karyotypic evolution within Vespertilionidae

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Summary. The karyotypes of each 2 species of Nyctalus and Murina are examined. It is assumed that the diploid number of vespertilionid ancestor was 44 with a fundamental number of 50 and that the mechanism of karyotypic evolution within subfamily Vespertilioninae is mainly caused by centric fusion. On the other hand, the karyotypic alteration of subfamily Murininae may be evolved by non-Robertsonian translocation.

Some authors[3–6] have suggested that the diploid number (2n) of vespertilionid ancestor was between 44 and 50 with a fundamental number (FN) of 50, and that karyotypic alteration of the majority of genera in Vespertilionidae was mainly evolved by centric fusion. In this paper, we discuss the hypothetical karyotype of vespertilionid ancestor and suggest mechanisms of karyotypic evolution in this family, and compare the karyotypes of some vespertilionid bats studied by us with those of other species reported so far. The technique used in this study was that described by Uchida and Andō[7]. For chromosomal classification the method of Patton[8] was adopted. Karyotypes of 16 Japanese species are listed in the table, and out of them the karyotypes of 6 species are represented in figure 1. In figures 2 and 3 are shown respectively the karyotypes of 2 Japanese noctule bats (Nyctalus furvus and N. lasiopterus) and 2 Japanese tube-nosed bats (Murina aurata and M. leucogaster) whose karyotypes are reported for the first time or not yet in detail.

From the facts, as shown in the table and figure 1, and as already described by some authors[3–6], that the FN values are more constant than the 2n ones, it is assumed that the mechanism considered responsible for karyotypic evolution in this family is mainly Robertsonian translocation (centric fusion or fission) which leads to formation of biarmed elements from uniarmed ones or its reverse. Some bat workers[9–11] suggested on the basis of morphological characters that the vespertilionid ancestor might be a Myotis-like bat. Taking their opinions into consideration, it seems that the karyotypic evolution in this family is mainly attributed to centric fusion.

A case of centric fusion may be found within genus Nyctalus. The karyotype of N. furvus (figure 2a) resembles closely to those of Myotis (figure 1a). And similar karyotypes are found in European species of Pipistrellus (e.g., P. nathusii, P. kuhli and P. savii)[12–15], too. Con-

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[1] Acknowledgment. The authors are very grateful to Prof. T. Wasano of Fukuoka University (Professor emeritus of Kyushu University) for the encouragement, Mr Harada for the technical assistance, and Prof. E. W. Jameson, Jr, University of California for comments on the manuscript.

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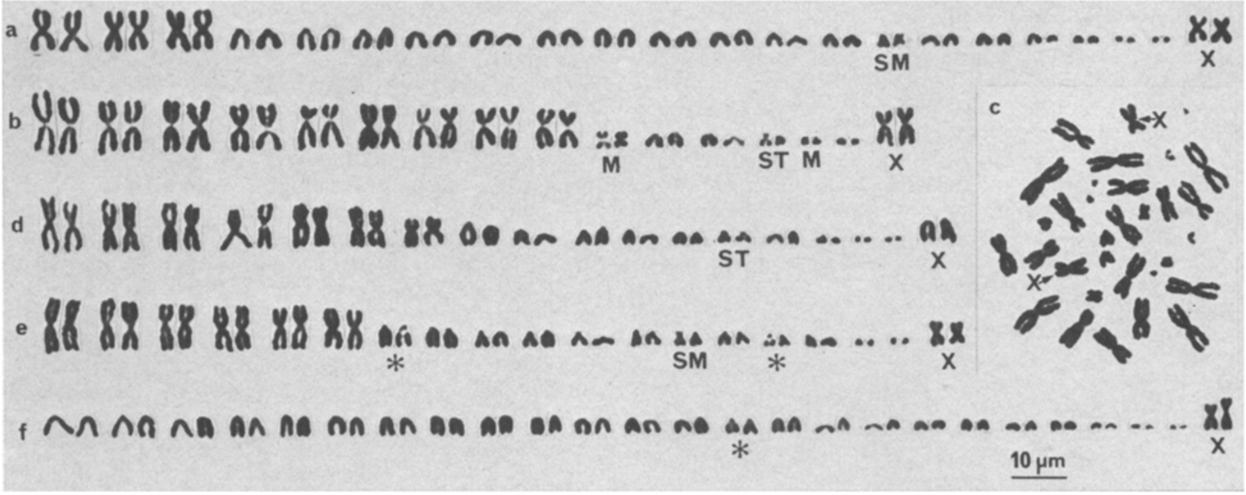

Fig. 1. Representative karyotypes of 6 vespertilionid species (females). a) Myotis nattereri; b) Plecotus auritus; c) Barbastella leucomelas; d) Pipistrellus endoi; e) Vespertilio orientalis; f) Eptesicus japonensis. M = Metacentrics; SM = submetacentrics; ST = subtelocentrics; * = marker chromosomes (autosomes having secondary constriction).

sidering that Pipistrellus might have been derived from a Myotis-like bat and the former may be more specialized than Myotis, and that Nyctalus is placed as a member of Pipistrelloid genera and is considered to be a direct offshoot of Pipistrellus[11], it is presumed that the ancestral form of Pipistrellus had the karyotype of 2n = 44 with a FN = 50 at the time of divergence from Myotis-like form, and its karyotype has been inherited by some living pipistrelle bats even now, and that Nyctalus also differentiated in a similar way from a stem of Pipistrellus without a great change of the karyotype. Therefore, we assume the karyotype of N. furvus to be rather primitive among those of Nyctalus examined so far[16,17]. The karyotype of N. lasiopterus (figure 2b) is strikingly similar to that of N. noctula[16]. In comparison with the karyotype of N. furvus and those of the above 2 species, the autosomal elements of N. lasiopterus and N. noctula are more by one pair of large M · SM-autosomes and are fewer by 2 pairs of large or medium-sized A-ones than those of N. furvus. The one additional biarmed pair found in N. lasiopterus and N. noctula may have originated, as a result of centric fusion, from certain 2 pairs of uniarmed autosomes such as those found in N. furvus.

As pointed out previously, a hypothetical karyotype of verspertilionid ancestor was described by some authors[3-6]. However, unless the karyotypes of the members of Murina are studied and compared with those of other verpertilionid bats, it is premature to estimate what karyotype had vespertilionid ancestor possessed, because Murina has been placed in subfamily Murininae of this family and morphological taxonomists[10,18,19] using traditional procedure have described that this bat is a local and specialized offshoot from some low, Myotis-like vespertilionine form. When one surveys the karyotypes of M. aurata and M. leucogaster (figure 3), 4 pairs of large to medium-sized ST-autosomes in the 2 species appear to have small secondary arms. We regarded these autosomes as subtelocentrics, but it is still open to question whether the 4 pairs of autosomes are satisfactory as biarmed ones. Therefore, the FN of 2 species are given here as tentative values (table). The karyotypes of 2 species in Murina have the same 2n value, but a slight difference is recognized between them in FN value (60 and 58) and in shape of a medium-sized autosomal pair (SM-autosomes in M. aurata, but ST-ones in M. leucogaster) (figure 3a, b). Harada[20] reported the karyotype of M. leucogaster to be 2n = 44 and FN = 50, differing from our data in FN value. This difference is due to that

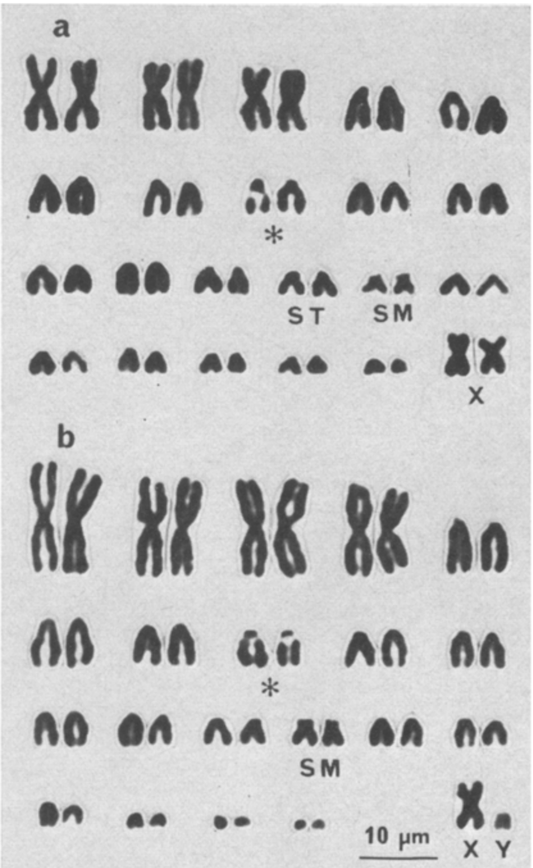

Fig. 2. Representative karyotypes of a female Nyctalus furvus (a) and a male Nactalus lasiopterus (b). SM = Submetacentrics; ST = subtelocentrics; * = marker chromosomes (autosomes having secondary constriction).

we regarded the 4 pairs of autosomal elements, which he judged to be acrocentric, as subtelocentrics, but not geographical variations as found in the karyotypes of Uroderma[21] and Macrotis[22].

In comparing the karyotypes of Murina (figure 3) and those of Myotis (e.g., figure 1a), the 2n of each genus has the same value, but in the autosomal elements of the former, as described above, there are 4 pairs of ST-autosomes having small secondary arms which cannot be found in the autosomal ones of the latter. For explanation of karyotypic alteration in Murina, thus, it is necessary to consider the chromosomal changing mechanisms other than the Robertsonian translocation, i.e., pericentric inversion or growth of heterochromatic arm[23]. But the karyotypes of Murina have essentially many similarities to those of Myotis. According to their systematic allocations, it seems that the karyotype of Murina originated from that of Myotis, and that the autosomes having small short arms which cannot be found in autosomal elements of Myotis are evolved by non-Robertsonian translocation of large to medium-sized uniarmed ones.

Collating the karyotypic characteristics of vespertilionid bats with their taxonomic remarks, we may conclude that the karyotypes of the majority of genera within subfamily Vespertilioninae of this family, except for that of Eptesicus (figure 1f), are mainly differentiated from that of Myotis by centric fusion, although slight karyotypic variations which cannot be explained by Robertsonian translocation are found within many genera. It seems, however, that the directions of karyotypic evolution in Miniopterus (subfamily Miniopterinae)[24] and Murina (subfamily Murininae) have independently each other gone forward different courses from the direction of vespertilionine bats, namely Miniopterus has tended to increase the 2n by centric fission and Murina has increased the FN by non-Robertsonian translocation respectively, although their karyotypes originated substantially from Myotis-like one.

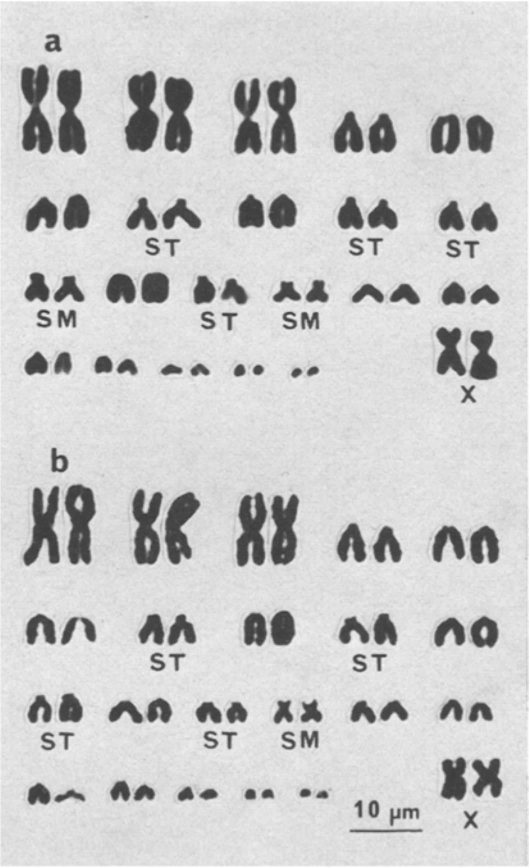

Fig. 3. Representative karyotypes of a female Murina aurata (a) and a female Murina leucogaster (b). SM = Submetacentrics; ST = subtelocentrics.

Comparison of the karyotypes among 16 Japanese vespertilionid bats

Species	2n	Autosomes			Sex chromosomes		FN
		M,SM	ST	A	X	Y	
Subfamily Vespertilioninae							
Genus Myotis							
M. nattereri	44	4	0	17	SM	–	50
M. hosonoi	44	5	0	16	SM	A	52
M. frater	44	5	0	16	SM	SM	52
M. macrodactylus	44	5	0	16	SM	SM	52
Genus Plecotus							
P. auritus	32	11	1	3	SM	A	54
Genus Barbastella							
B. leucomelas	32	10	0	5	SM	A	50
Genus Pipistrellus							
P. endoi	36	7	1	9	A	A	50
P. abramus	26	8	2	2	A	A	44
Genus Nyctalus							
N. furvus	44	4	1	16	SM	–	52
N. lasiopterus	42	4	1	15	SM	M	50
Genus Vespertilio							
V. superans	38	7	0	11	SM	A	50
V. orientalis	38	7	0	11	SM	A	50
Genus Eptesicus							
E. japonensis	50	0	0	24	SM	SM	48
Subfamily Murininae							
Genus Murina							
M. aurata	44	5	4	12	SM	A	60(?)
M. leucogaster	44	4	4	13	SM	A	58(?)
Subfamily Miniopterinae							
Genus Miniopterus							
M. schreibersi	46	4	0	18	SM	A	52

2n = Diploid number; M = metacentrics; SM = submetacentrics; ST = subtelocentrics; A = acrocentrics; FN = fundamental number.

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